SUPPLEMENTARY INFORMATION

Anopheles and *Plasmodium*: from laboratory models to natural systems in the field

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Supplementary Methods

Mosquito strain

The Yaoundé mosquito strain was established in 1988 from field specimens caught in the suburbs of Yaoundé city (in the South of Cameroon). Mosquitoes were adapted to feed through a parafilm membrane and then maintained under standard insectary conditions . The colony belongs to the M molecular and Forest chromosomal forms (standard chromosomal arrangements).

P. falciparum gametocyte carriers recruitment

P. falciparum gametocyte carriers were recruited among five to twelve-year old children at schools in Mfou, a town located 30 km from Yaoundé city in an area endemic for malaria. Blood samples were collected by finger-prick from each volunteer and thick blood smears were Giemsa-stained and examined by microscopy for the presence of *P. falciparum*. Sexual and asexual stages were counted in observed fields that cumulatively contained at least 500 leucocytes; an estimate of parasite density was obtained by assuming a standard number of 8000 leucocytes/µl of blood. Children with asexual parasitaemia exceeding 1,000 parasites/µl were immediately treated with amodiaquine and artesunate drug combination according to national guidelines. Asymptomatic gametocyte-positive children were enrolled as volunteers. The recruitment procedures were approved by the Cameroonian and WHO ethical review committees.

Mosquito infections, RNA isolation and transcript level analysis

Females of the Yaoundé *A. gambiae* strain were fed either on gametocyte-positive blood or non-infected human blood. The Yaoundé strain is representative of the local natural transmission system in South Cameroon, where malaria transmission is mainly due to *A.*

gambiae (M and S molecular forms, Forest chromosomal form), although *Anopheles funestus*, *Anopheles nili* and *Anopheles moucheti* can also be locally important (Antonio-Nkondjio *et al*, 2002).

Transcript level analysis of *CTL4*, *CTLMA2* and *LRIM1* was carried out using quantitative RT-PCR (QRT-PCR). Briefly, midguts and carcasses of Yaoundé mosquitoes, fed on naïve or *P. falciparum* infected human blood, were dissected 24 h post blood feeding, and were kept separately in RNAlater (Ambion) before RNA isolation. A batch of thirty control mosquitoes was dissected seven days post blood feeding to determine the level of infection. Total RNA was isolated using the TRIzol Reagent (Invitrogen) according to the supplier's instructions, and contaminant genomic DNA was removed by DNase I treatment. cDNA was synthesized from total RNA (2-3 μg) using the SuperScript II RNase H^- Reverse Transcriptase and oligo$(dT)_{12\text{-}18}$ as described by the supplier (Life Technologies, Inc.). Quantitative RT-PCR (QRT-PCR) was performed with the ABI Prism 7700 Sequence Detection System, using the SYBR Green PCR Master Mix kit (Applied Biosystems) according to the manufacturer's instructions. Primer sequences are described by Osta *et al* (2004). Relative gene expression values were calculated using the Comparative C_T Method after checking for efficiency of target amplification as described in User Bulletin #2. The S7 ribosomal protein gene was used as internal reference.

Supplementary references:

Antonio-Nkondjio C, Awono-Ambene P, Toto JC, Meunier JY, Zebaze-Kemleu S, Nyambam R, Wondji CS, Tchuinkam T, Fontenille D (2002) High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. *J Med Entomol* **39:** 350-355

Osta MA, Christophides GK, Kafatos FC (2004) Effects of mosquito genes on *Plasmodium* development. *Science* **303:** 2030-2032